

Figure 1. Hypersensitivity of Gyrase Mutant Alleles

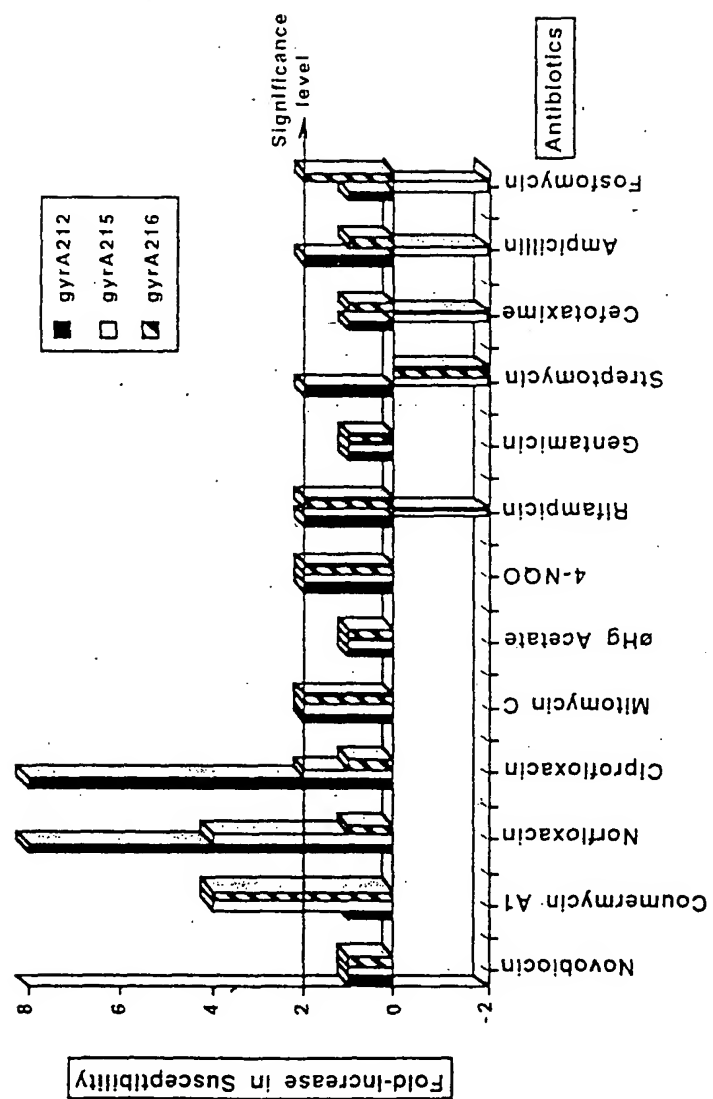


Figure 2. Hypersensitivity Profiles of *Salmonella* Ts Mutants

Gyrase Inhibitors			DNA/RNA metabolism				Protein metabolism				Cell wall inhibitors			
	Nov	Cou	Cipro	Nor	MitoC	gHg	NQO	Rif	Gen	Strep	Phen	Cefo	Amp	Fosfo
5155 dnaE	-	-	-	-	-	-	-	-	-	-	-	-	-	4
gyrA216	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gyrA215	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gyrA212	-	-	-	-	-	-	-	-	-	-	ND	-	-	-
7784 parC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
clm?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
parE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
parE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
parF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
parF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
parF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
clm?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
murB	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Round	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7587 dpa	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5110 mutS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50811 Thy	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7585 Odd	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5208 fish	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7141 Filam	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5052 Filam	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5051 Filam	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5041 UV	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5086 UV	-	-	-	-	-	-	-	-	-	-	-	-	-	-
clm?	-	-	-	-	-	-	-	-	-	-	-	-	-	-

.. indicates that there was no significant difference with the wild type parent strain. "ND": Not determined.

Abbreviations: Nov, novobiocin; Cou, coumermycin; Cip, ciprofloxacin; Nor, norfloxacin; MitoC, mitomycin C; gHg, phenylmercuric acetate; NQO, 4-nitroquinoline oxide; Rif, rifampicin; Gen, gentamicin; Strep, streptomycin; Phen, phenol; Cef, cefotaxime; Amp, ampicillin; Fosfo, fosfomycin; clm?, unknown conditional lethal mutant; Round, round cell morphology; Thy inc-, defective thymidine incorporation phenotype; Odd, odd cell shape morphology; Filam, filamentous cell morphology; UV-, UV sensitive. Known or closely related genotypes: *thrA*, DNA polymerase III α subunit; *gyrA*, gyrase subunit A; *parC*, GyrA-like subunit of Topoisomerase IV; *parE*, GyrB-like subunit of Topoisomerase IV; *parF*, Acetyl transferase activity, associated with the topoisomerase IV gene; *murB*, UDP-N-acetylglucosaminyl-3-enolpyruvate reductase; *dpaA*, Dihydrodipicolinate synthase; *murCEFG*, near cluster of L-Ala, DAP, D-Ala-D-Ala and NAG ligases; *fisH*, maybe *fisH* by map location.

Figure 3. Potential Relationships Among Essential Genes

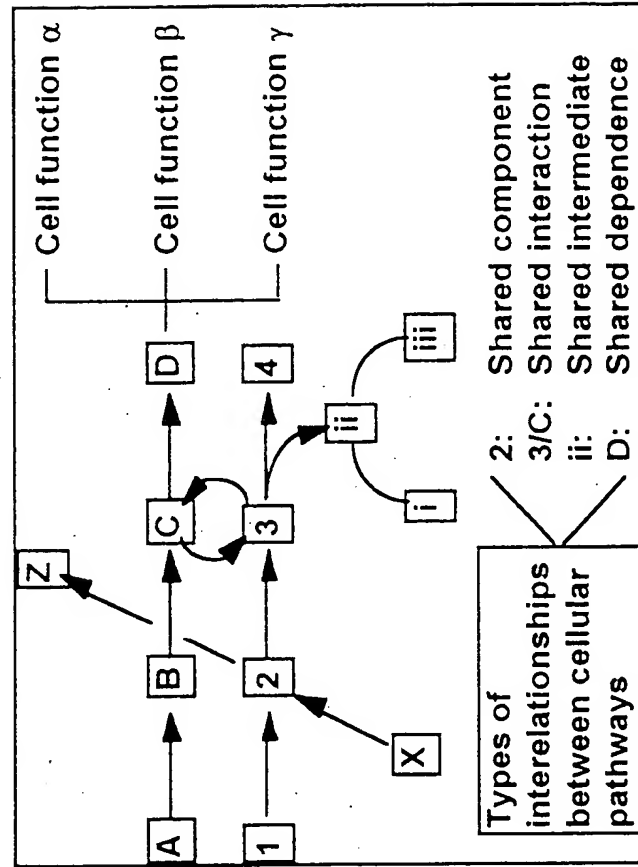


Figure 4. Single and Multi-Score Multichannel Screen

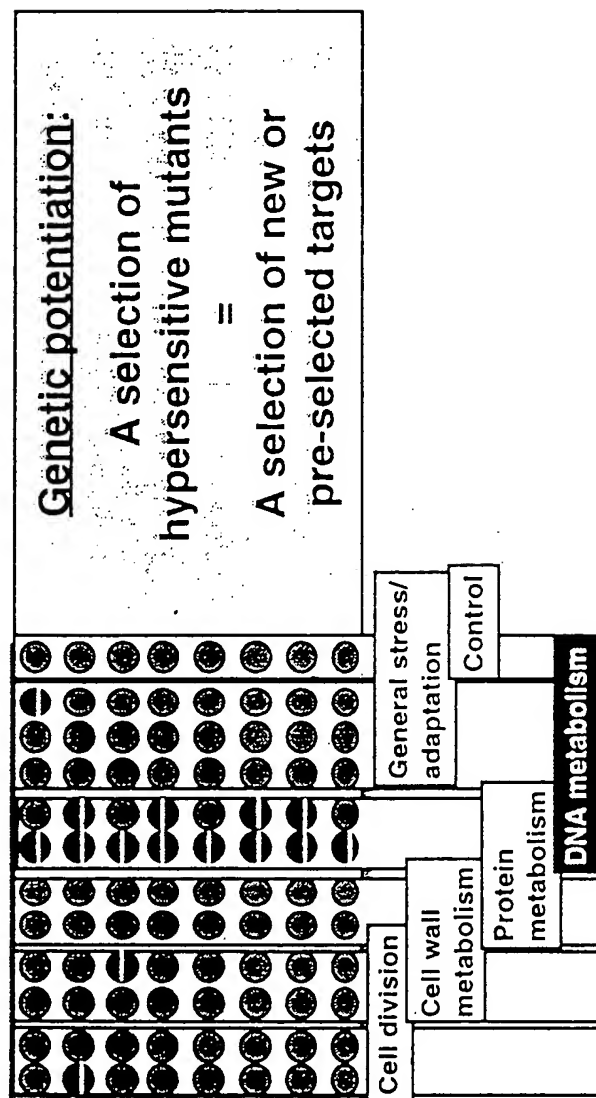


Figure 5. Operational Design of Multichannel Screen

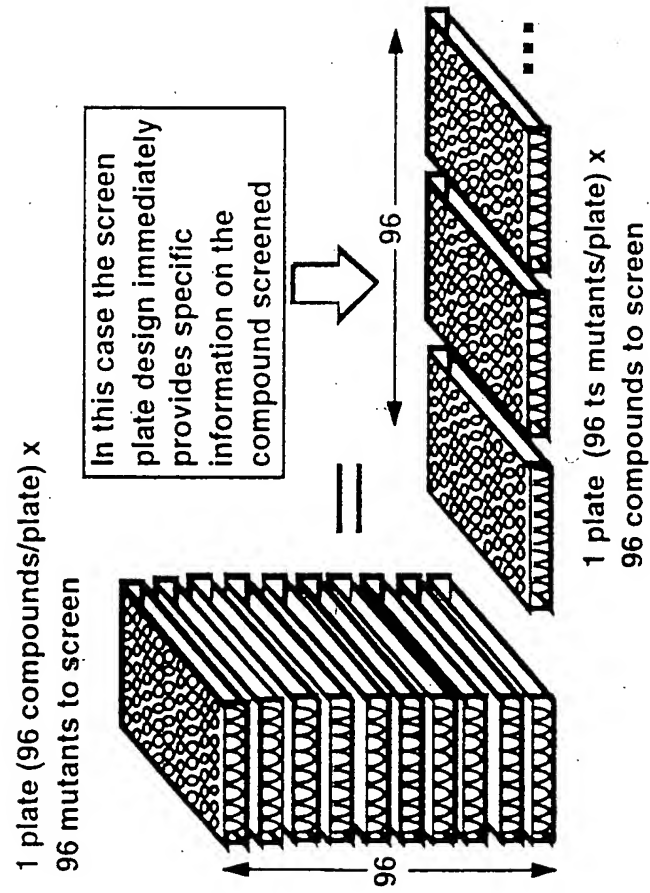
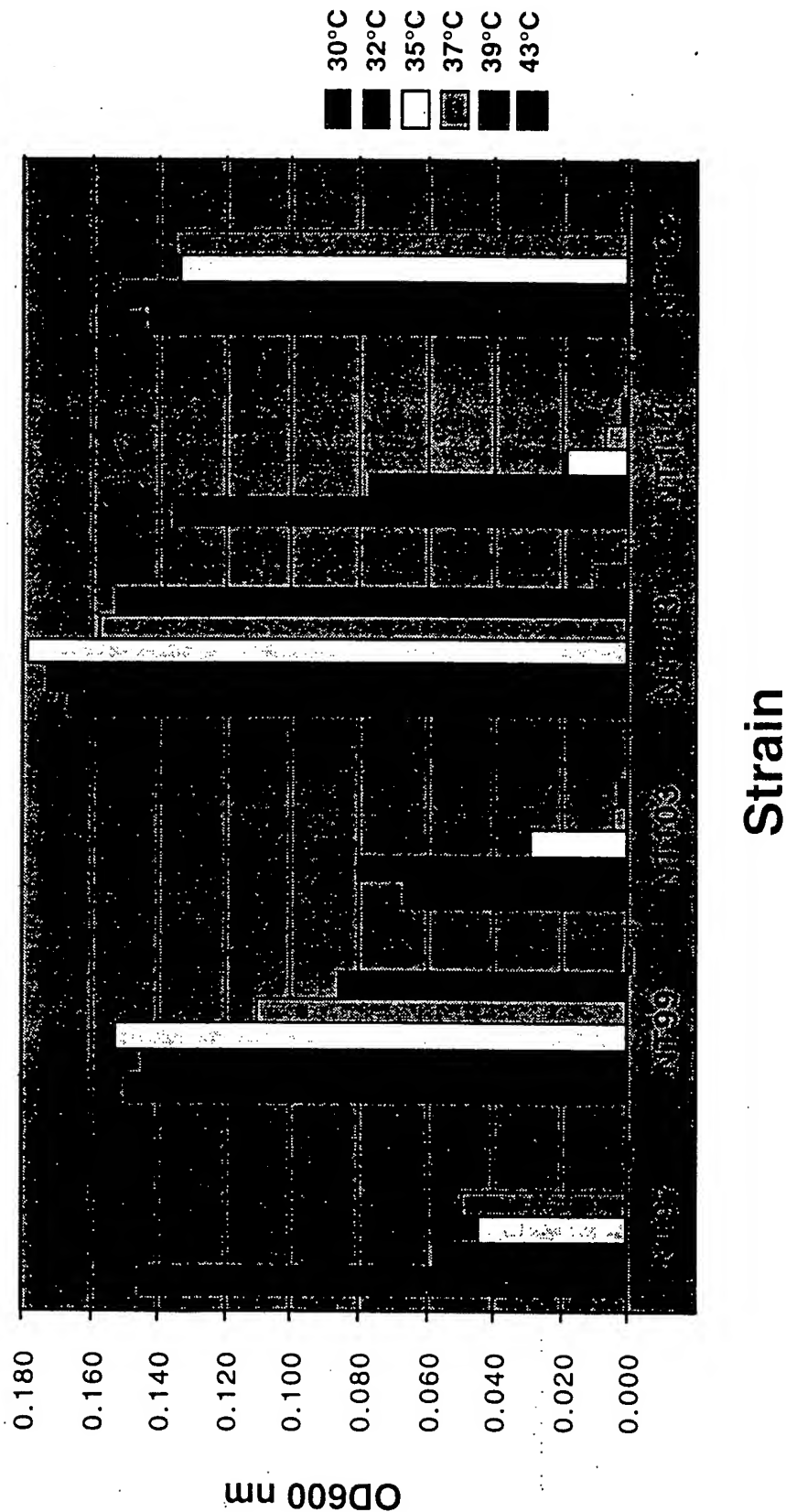


Fig 6

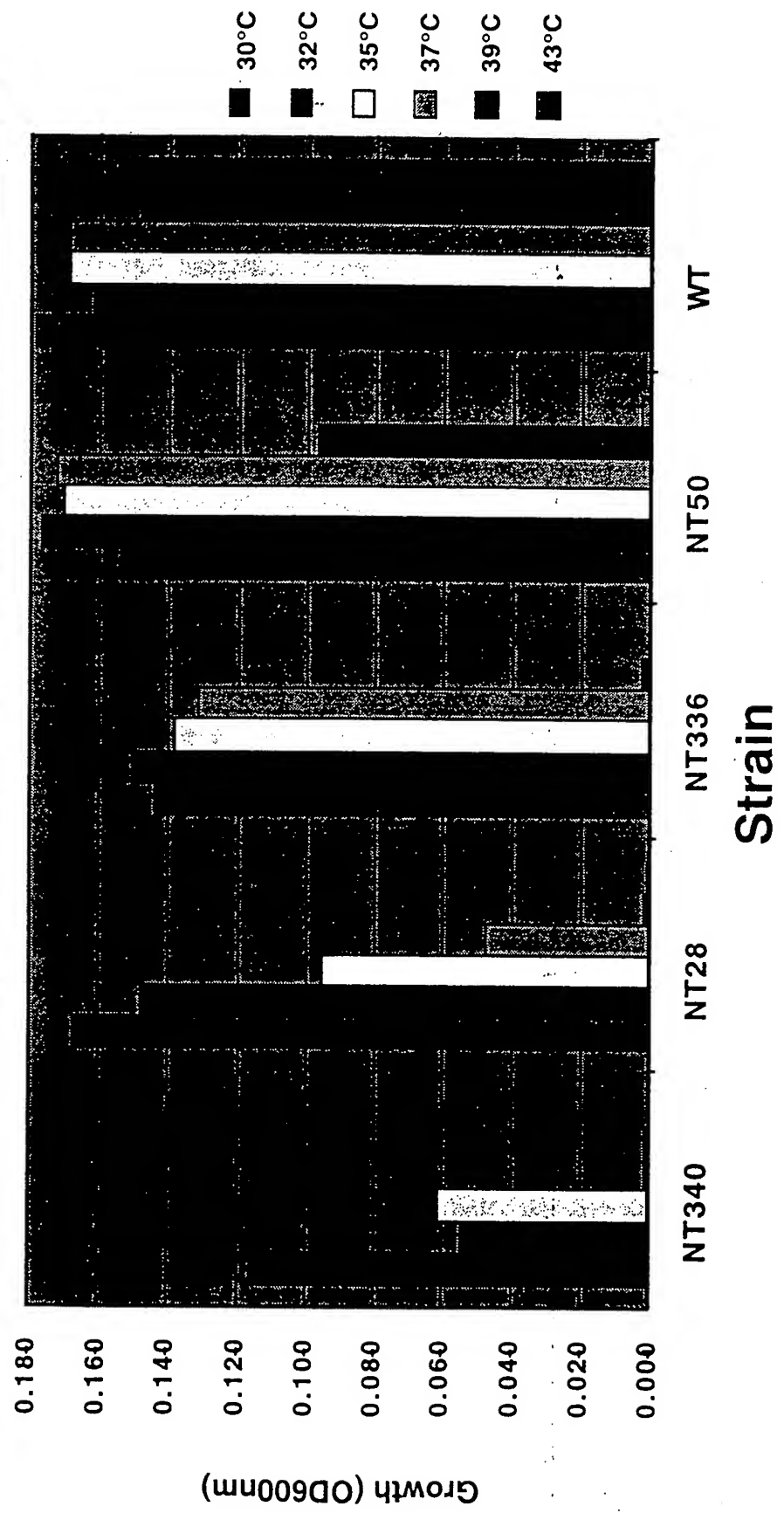
Heat-sensitivity Profiles of Ts Mutants in Different Genes



base 11

Fig 7

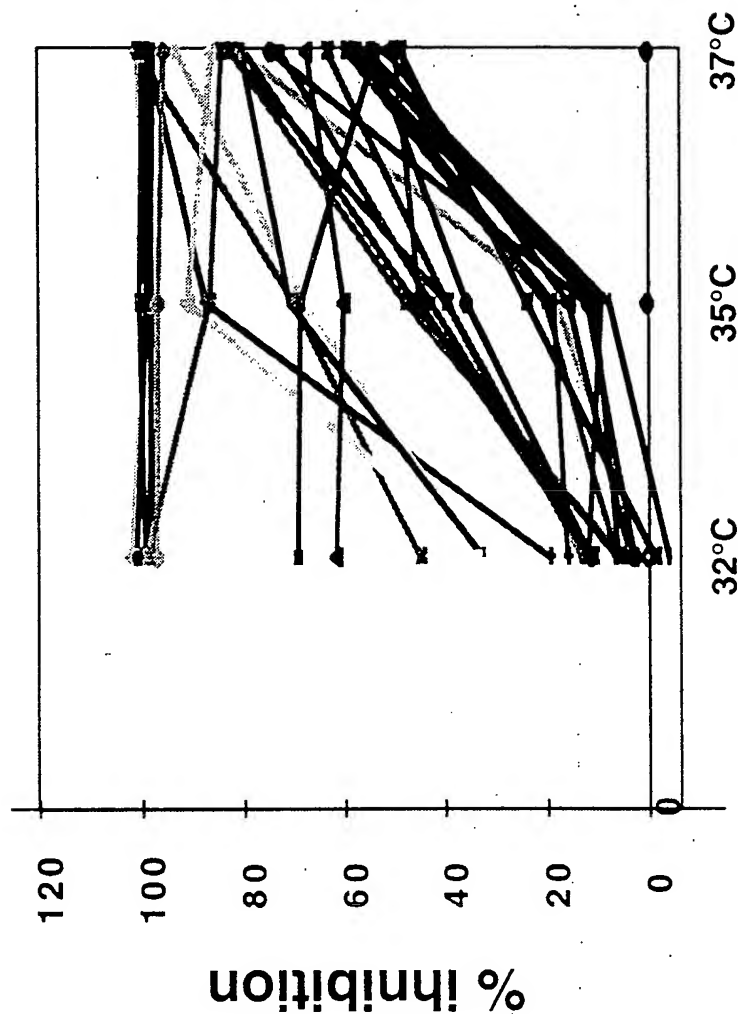
Heat-sensitivity Profiles of Different *po/C* Ts Alleles



mic

Fig 8

Temperature-dependent Hypersensitivity



Strain: NT 99

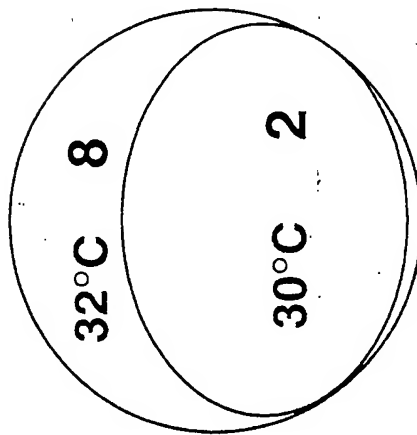
Inhibitory effect of
30 compounds at
32°C, 35°C & 37°C

450

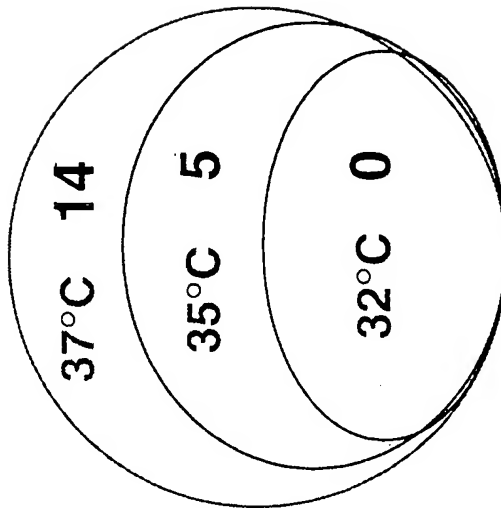
Fig 9

Hit criteria at different temperatures

Hit criteria: compd that inhibits mutant by $\geq 50\%$, and % inh.
on mutant is higher than on WT by $\geq 30\%$
of compounds tested: 480 for NT99; 240 for NT340



NT340



NT99

Fig 10

Concentration Control of Hit Rate

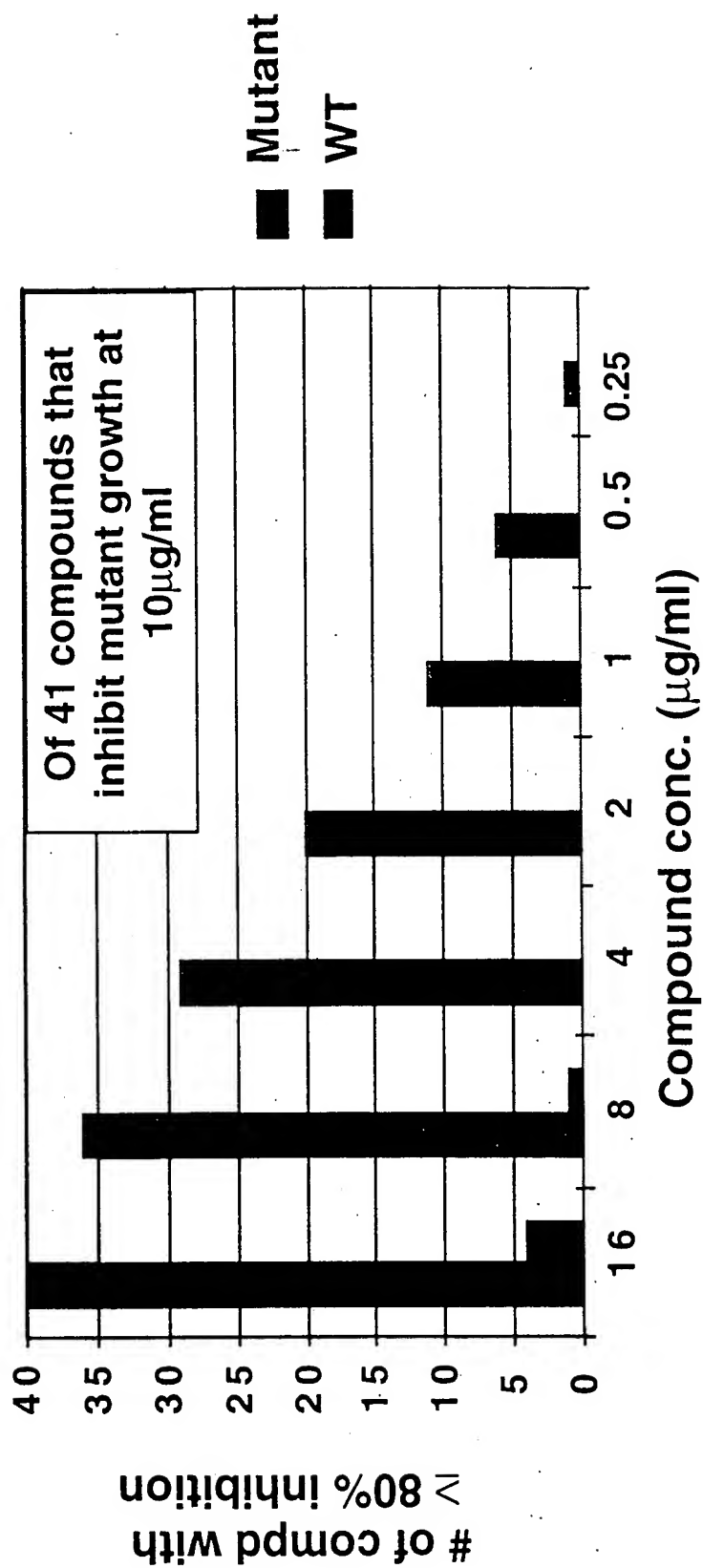
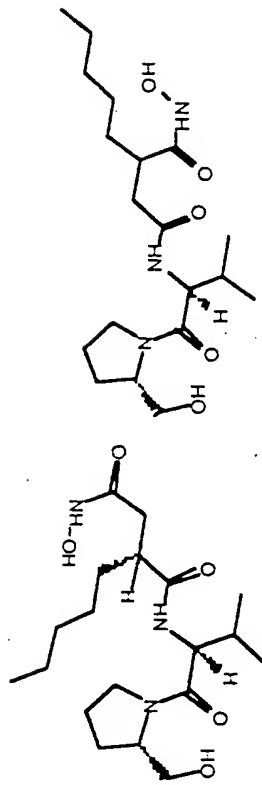


Fig 11 Structural Similarity of Compounds having
Identical Mutant Inhibition Profiles.



30-0014

20-0348

Fig 12 Pilot Screen With Unknown Inhibitors Against *S. aureus**

NT	20-0157	10-0617	50-0116	20-0204	20-0860	20-0123	10-0287	20-0045	10-0373	10-0752	20-0197	10-0797	10-3775	10-9370	10-9370
1														4	
2															
3															
4														4.8	
5															
6															
7															
8														ND	
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
32															
33															
34															
35															
36															
37															
38															
39															
40															
41															
42															
43															
44															
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															
93															
94															
95															
96															
97															
98															
99															
100															
101															
102															
103															
104															
105															
106															
107															
108															
109															
110															
111															
112															
113															
114															

*ND: No data available; blank boxes show no significant difference in MIC from the wild type strain (significance level > +/- 2-fold).

Fig 13 Anticipated Mutant Inhibition Profiles

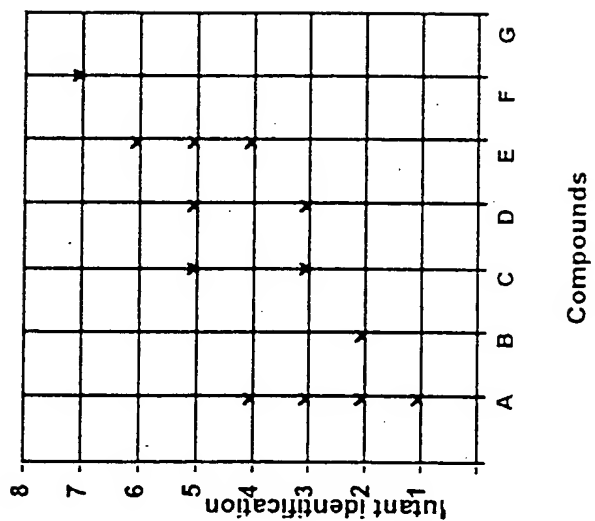


Fig 14 Hit Rate per Multiple of Mutants

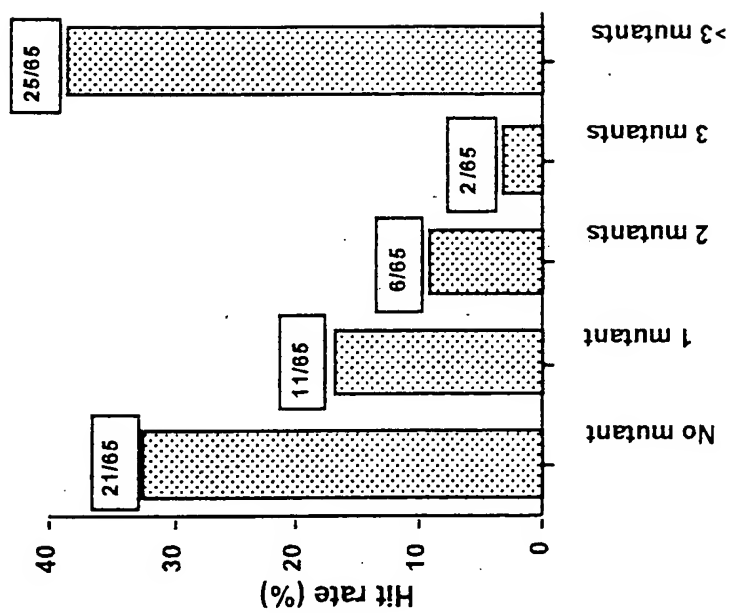


Fig 15 MIC Values of Hits Affecting Few or No Mutants

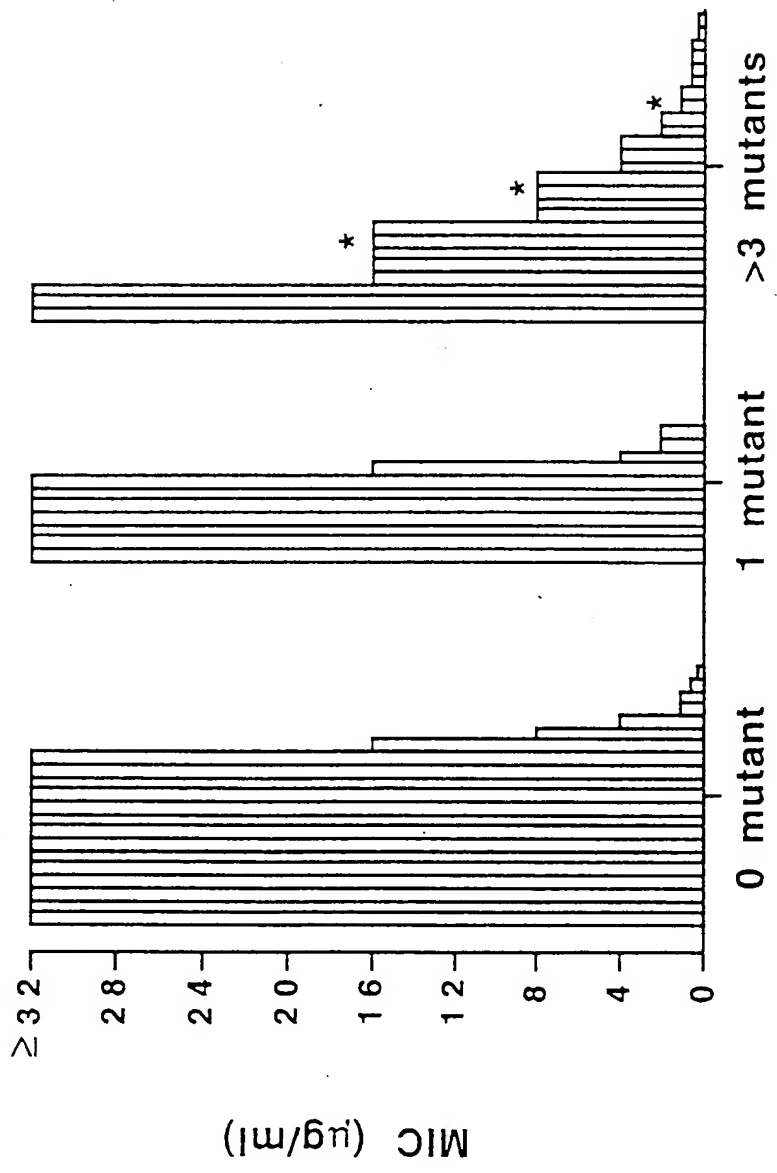


Fig. 16 Relative Number of Hits per Mutant

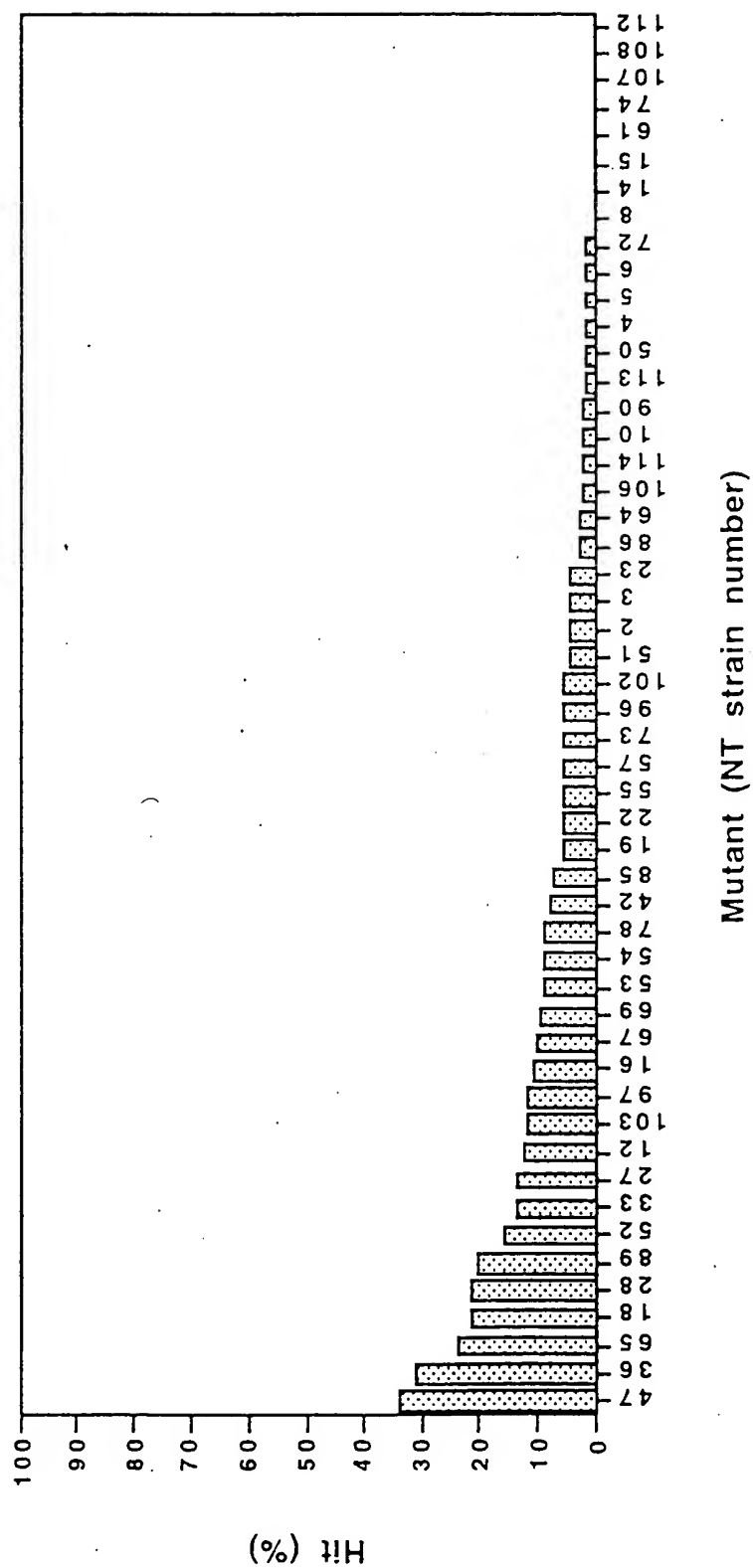


Fig. 17 Multichannel Screen Advantage

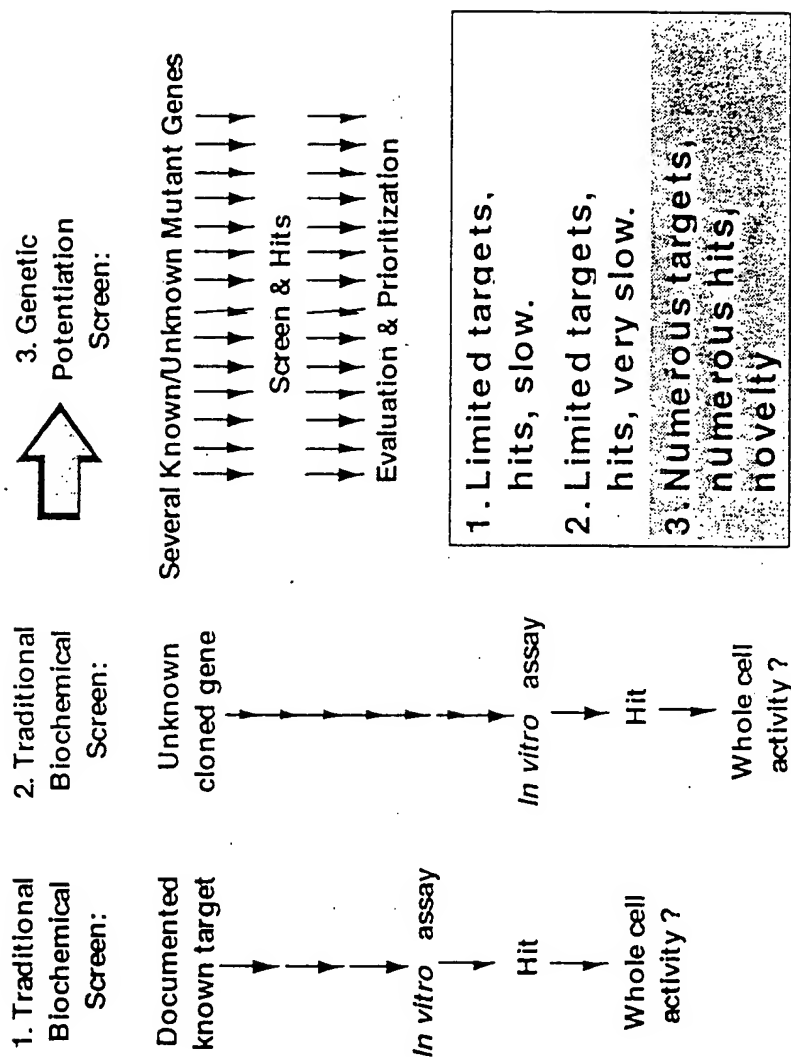


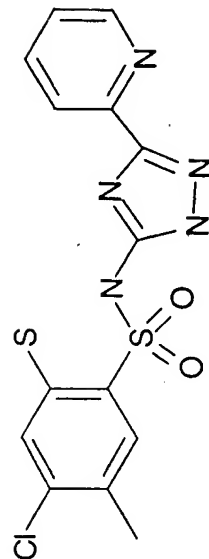
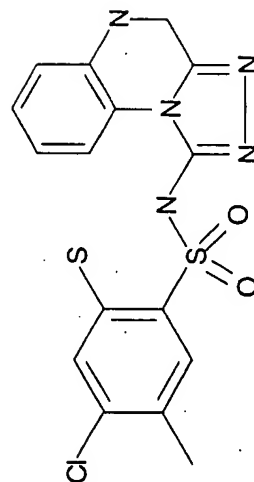
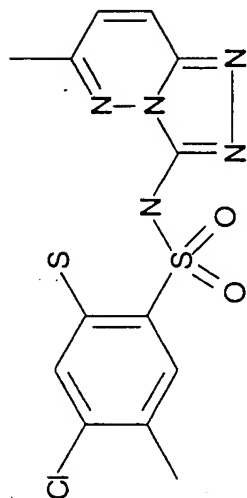
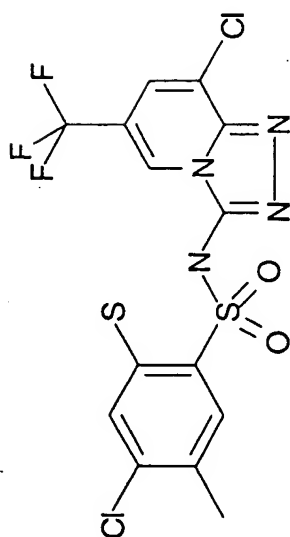
Fig 18 Strategy for Selection of Dominant Lethal Mutants

Relevant Genotype	Phenotype
ts/hypersens	No growth at high temperature
dom	No growth (not viable)
ts/hypersens dom	No growth (not viable)
ts/hypersens dom	Growth at high temperature No growth at low temperature

F-15 19

Structurally Similar Compounds Inhibit NT94

F-15 19



F-15 19

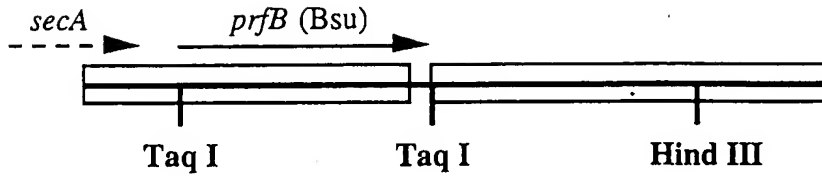
Fig. 20



Clone 0135-30 in 117ev
8/21/96

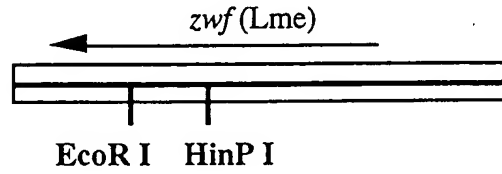
Fig 21 NT3

Fig 21



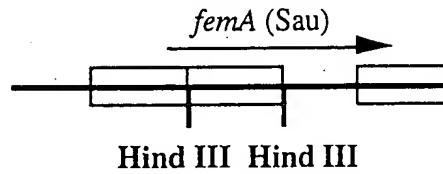
22 NT5

Fig 22



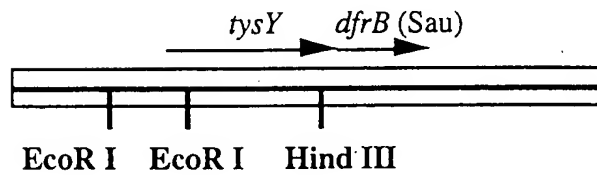
23 NT6

Fig 23



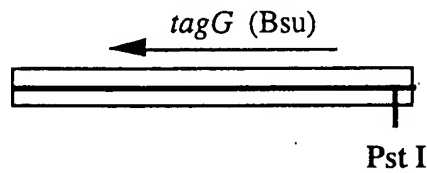
24 NT8

Fig 24



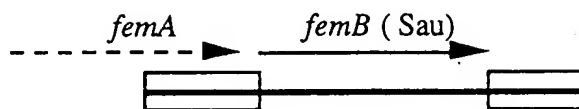
25 NT12

Fig 25



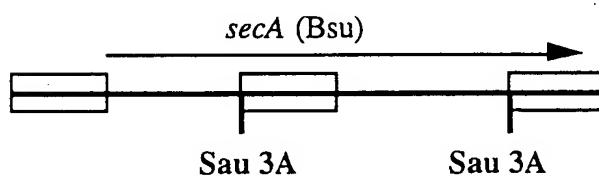
26 NT14

Fig 26



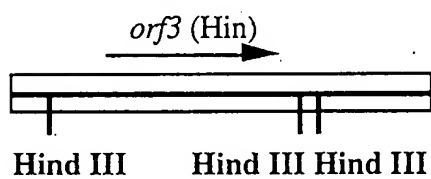
27 NT15

Fig 27



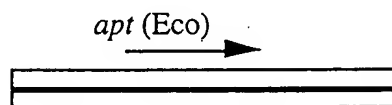
28 NT16

Fig 28



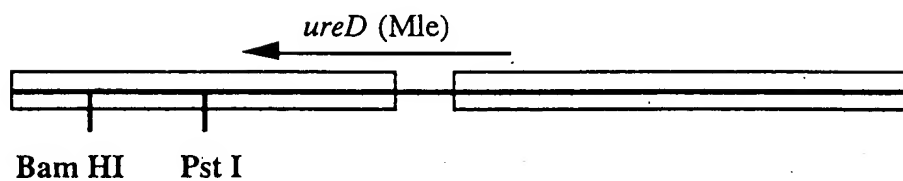
29 NT17

Fig 29

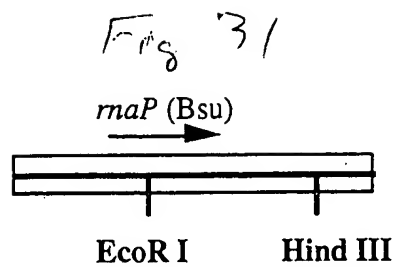


30 NT18

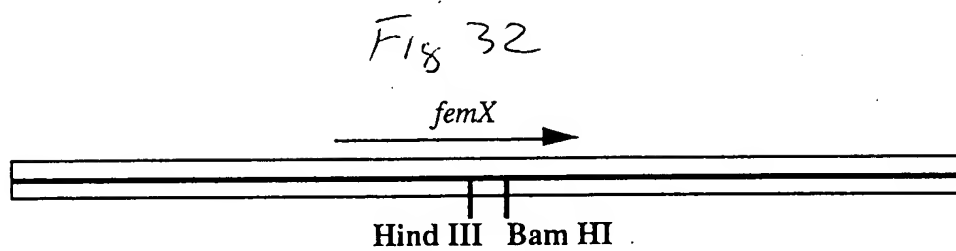
Fig 30



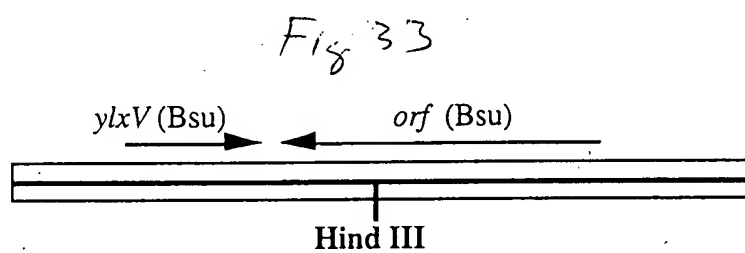
- 1 NT19



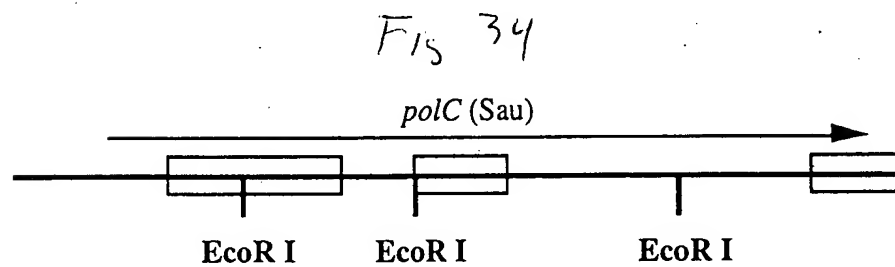
32 NT23



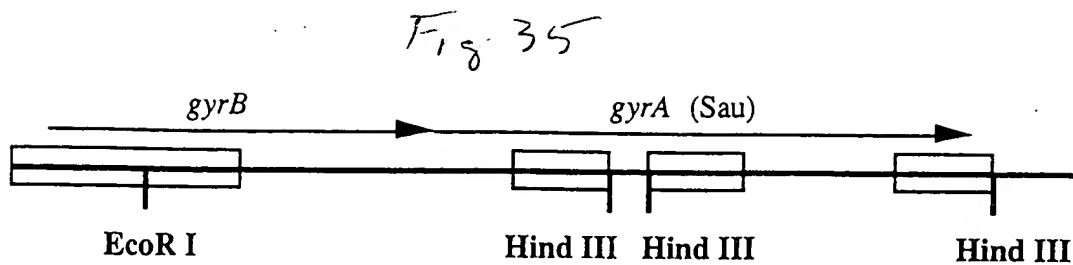
33 NT27



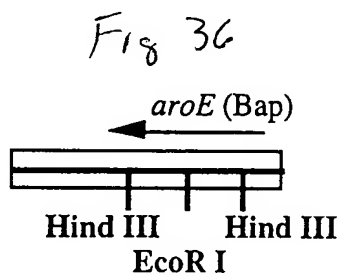
34 NT28



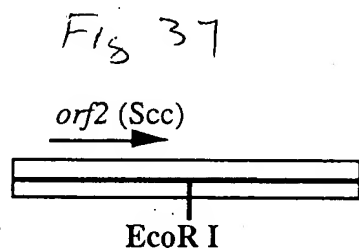
35 NT29



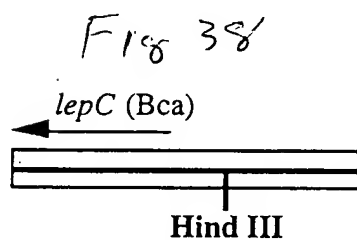
36 NT31



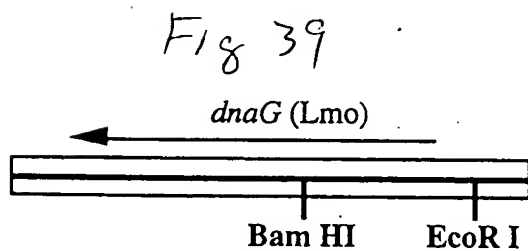
NT33a



38 NT33b

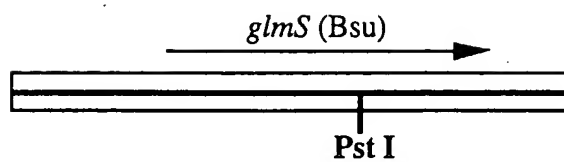


39 NT36



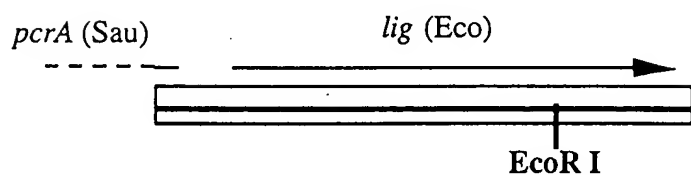
40 NT37

Fig 40



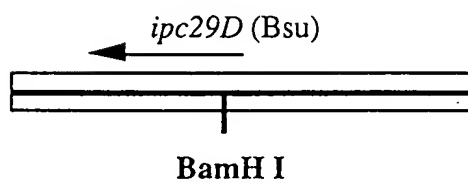
41 NT41/64

Fig 41



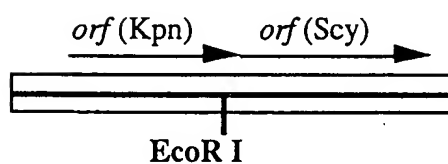
42 NT42

Fig 42



43 NT47

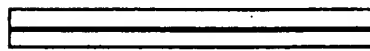
Fig 43



44 NT51

Fig 44

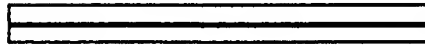
ycfB (Hin)



45 NT52

Fig 45

kimE (Mth)

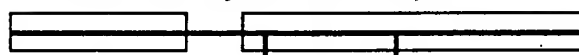


46 NT53

Fig 46

papS (Bsu)

birA (Bsu)



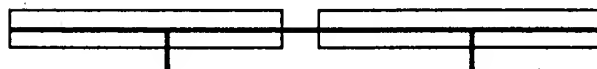
Pst I

Pst I

47 NT54

Fig 47

tagD (Bsu)



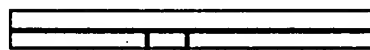
Hind III

EcoR I

48 NT55

Fig 48

nadE (Eco)

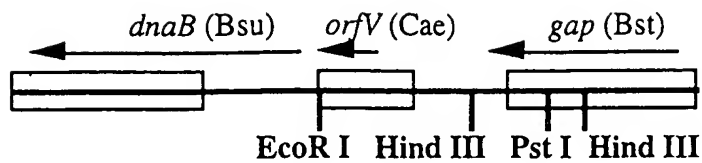


Hind III

Pst I

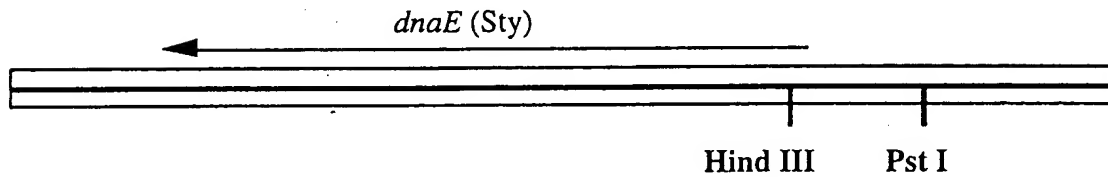
49 NT57

Fig 49



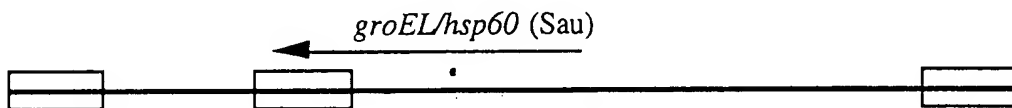
50 NT68

Fig 50



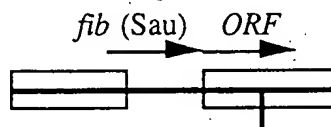
51 NT78

Fig 51



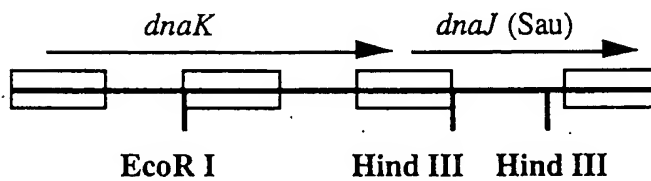
52 NT81

Fig 52



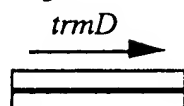
53 NT86

Fig 53



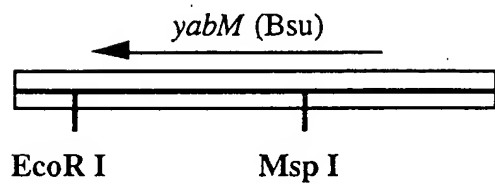
54 NT89

Fig 54



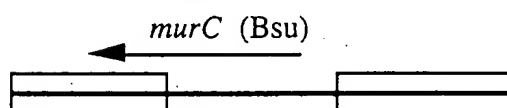
55 NT94

Fig 55



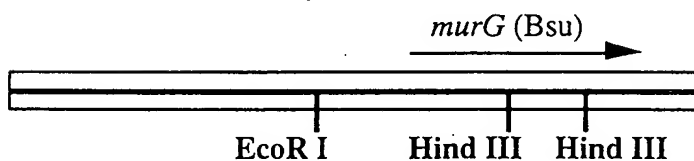
56 NT96

Fig 56



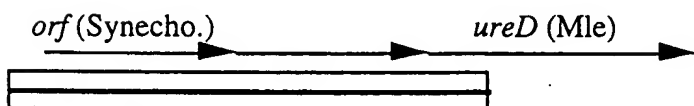
57 NT99

Fig 57



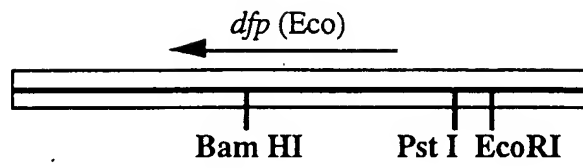
58 NT102

Fig 58



59 NT114

Fig 59



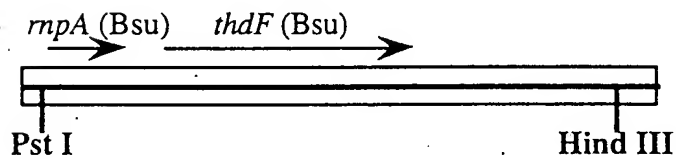
60 NT124

Fig 60



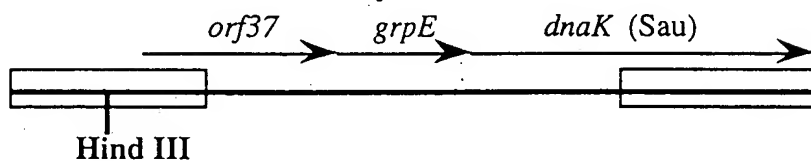
61 NT125

Fig 61



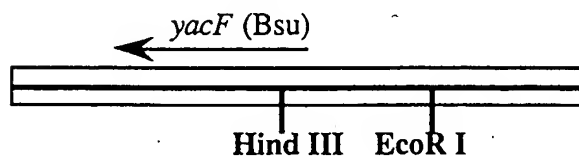
62 NT144

Fig 62



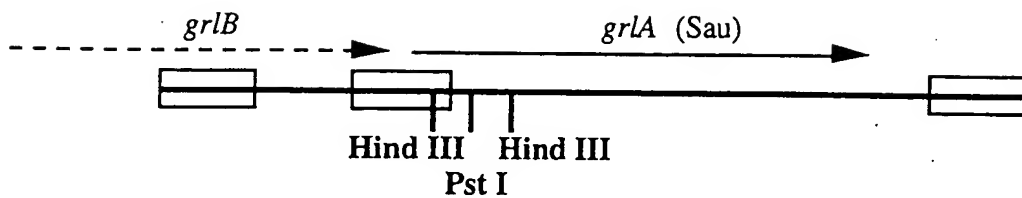
63 NT152

Fig 63



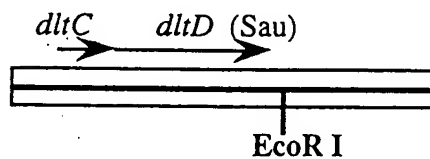
64 NT156

Fig 64



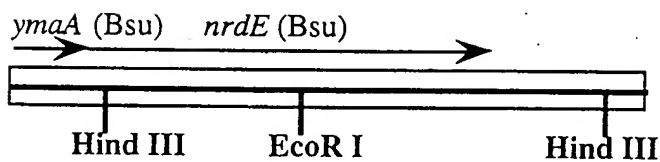
65 NT160

Fig 65



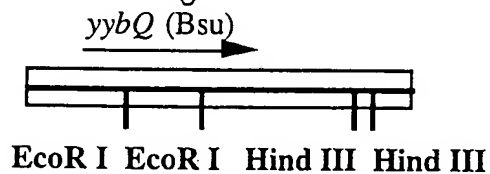
66 NT166

Fig 66



67 NT199

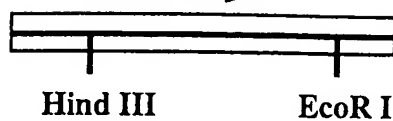
Fig 67



68 NT201

Fig 68

murB (Bsu)



69 NT304

Fig 69

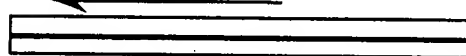
dod (Bsu)



70 NT310

Fig 70

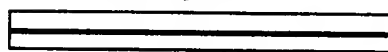
ddlA (Ehi)



71 NT312

Fig 71

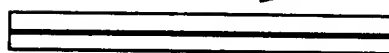
mg442 (Mge)



72 NT318

Fig 72

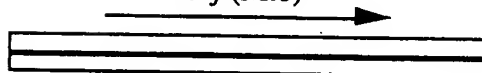
spoVC (Bsu)



73 NT321

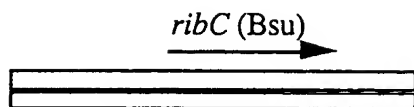
Fig 73

orf (Mtb)



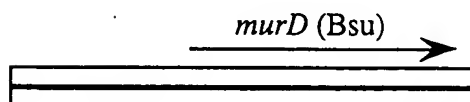
74 NT325

Fig 74



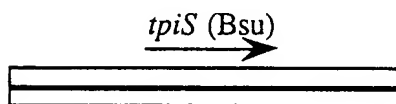
75 NT333

Fig 75



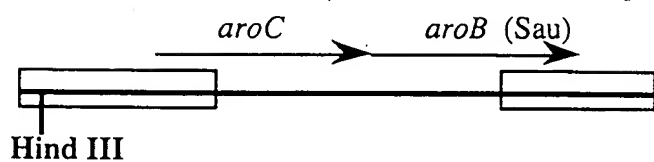
76 NT346

Fig 76



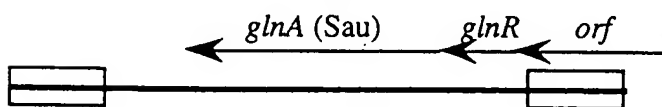
77 NT348

Fig 77

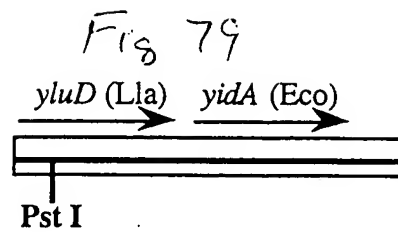


78 NT359

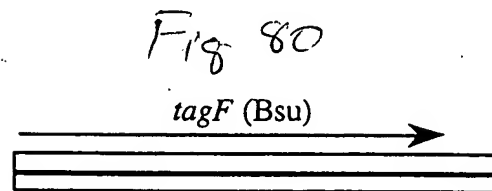
Fig 78



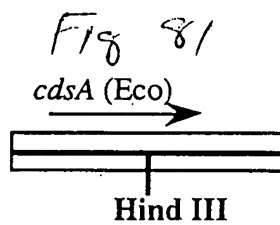
79 NT371



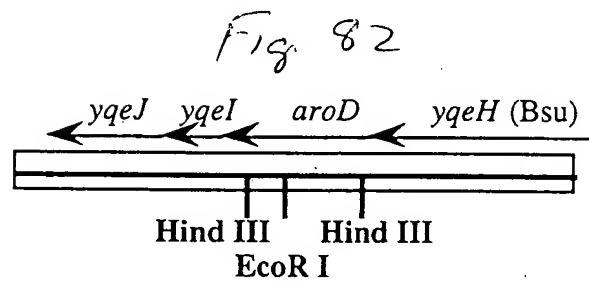
80 NT379



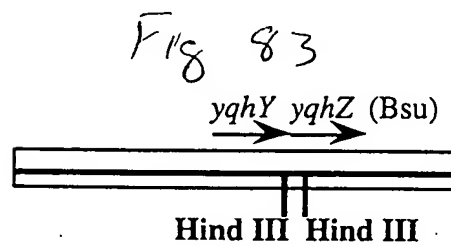
81 NT380



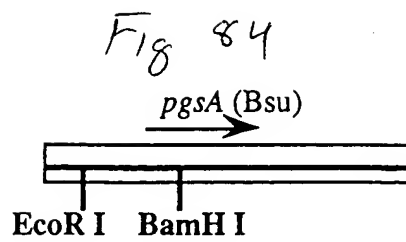
82 NT401



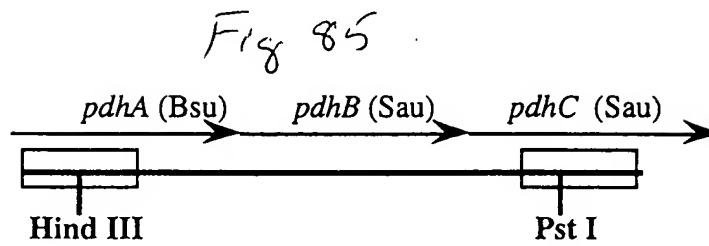
83 NT423



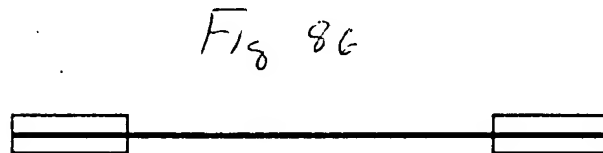
84 NT432



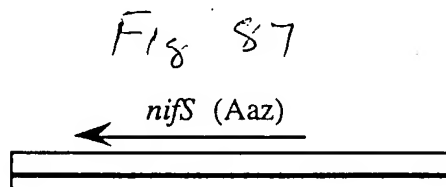
85 NT435



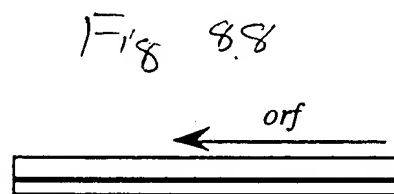
86 NT437



87 NT438

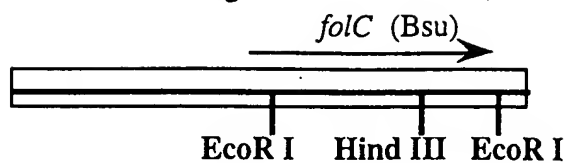


88 NT462



89 NT482

Fig 89



9.7 NT486

Fig 90

